PHYSICO-KINETICS OF THERMALLY PROCESSED AND CANNED CHILLI (*CAPSICUM ANNUM VAR KULAI)* IN THE FORM OF CHILLI PUREE

by

REVATHI A/P RAMACHANDRAN

Thesis submitted in fulfilment of the
requirements for the Degree
of Master of Science

DECEMBER 2005

To Gobithasan,

Your love, encouragements and patience has kept me going all these years.

ACKNOWLEDGEMENTS

Nobody writes a thesis without help, and I am not an exception. First of all, I thank God for the guidance in helping me to complete my research. I would like to express my gratitude to Associate Professor Dr. Noryati Ismail, for her comments, supervision and input, which helped make this research project a success. I also would like to thank Puan Wan Nadiah for her useful comments which helped to clarify some my doubts. Not forgetting the staffs of the Food Technology Division, especially Mr. Joseph Hemadry and Puan Siti, who provided helpful technicalities, advice and resources during the research.

Finally, in addition to these professional acknowledgements, I have to personally acknowledge my parents, in-laws, relatives and friends for their morale support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

FIZIKO-KINETIK CILI (Capsicum annum var Kulai) PURI YAND TELAH DIPROSES TERMA DAN DITIN

ABSTRAK

Pemprosesan terma cili merah (*Capsicum annum var Kulai*) dalam bentuk puri dan pengetinannya boleh menjadi satu proses alternatif untuk menghasilkan produk cili dalam bentuk puri yang akan mempunyai jangka hayat yang panjang. Selain daripada itu, sifat-sifat kimia cili yang memanfaatkan kepada pengguna juga dapat dipelihara. Cili merah dicelur pada suhu dan jangka masa yang berbeza di dalam 'steam jacketed kettle'. Sampel kemudiannya dikeluarkan, dibuang airnya, disejukkan lalu pemeriksaan dibuat keatas aktiviti enzim peroksida dan lipoxygenase. Keputusan menunjukkan deaktivasi enzim yang sempurna tercapai apabila cili dicelur pada suhu 100°C selama 6 minit.

Tiga jenis gam makanan (xanthan, low methoxyl pectin dan high methoxyl pectin) telah digunakan dalam kuantiti berlainan (0.5, 1.5 and 3.0% w/w) untuk mengkaji kesan suhu berlainan (70, 80 90 dan 100 °C) ke atas sifat reologi cili merah yang mengalami proses penyejatan selama 20 minit. Sifat reologi puri telah dikaji menggunakan Brookfield viscometer pada suhu yang disebut di atas. Adalah disimpulkan bahawa model 'Power Law' mewakili data tegasan ricih-kadar ricih dengan tepat. Kesandaran suhu terhadap kelikatan pada semua kadar ricih mengikuti hubungan Arrhenius dan tenaga aktivasi untuk aliran adalah diantara 20.15 dan 20.17 kJ/mol untuk HMP, 22.56 dan 28.77 kJ/mol untuk LMP dan 6.54 atau kurang untuk gam xanthan. Gam xanthan adalah paling kurang sensitif terhadap suhu dan juga memberikan kelikatan yang paling tinggi di antara ketiga-tiga hidrokoloid tersebut. Gam xanthan telah dipilih untuk meningkatkan kelikatan puri dalam kajian pemprosesan terakhir yang melibatkan proses penceluran,

pengkisaran, pengetinan dan pensterilan. Kinetik peringkat pertama berupaya menerangkan kadar kehilangan warna puri semasa diproses.

Degradasi warna puri tidak bersandar pada jenis gam dan kuantit yang telah digunakan. Kesan individu dan interaktif masa pemprosesan (15,30 dan 60 minit) dan kuantiti (0.5,1.5 and 3.0%) gam xanthan pada sifat reologi dikaji menggunakan kaedah permukaan sambutan (RSM) semasa proses sterilisasi. Adalah dilihat bahawa hanya masa pemprosesan yang memberi kesan terhadap degradasi warna manakala tiada kesan tersebut pada kelikatan dan kapasiti pegangan air. Kuantiti gam ($P < 0.05$) dan masa pemprosesan secara individu dan secara penggabungan memberi kesan terhadap nilai maut pemprosesan.

PHYSICO-KINETICS OF THERMALLY PROCESSED AND CANNED CHILLI (Capsicum annum var Kulai) IN THE FORM OF CHILLI PUREE

ABSTRACT

Thermal processing of red chilli (*Capsicum annum var Kulai*) in the form of puree, in cans can be an alternative process of producing a product with extended shelf-life while retaining its favourable qualities. Red chilli was subjected to blanching treatment at different temperatures and times in a steam jacketed kettle. Test samples were removed, cooled, drained and tested for enzyme activity (peroxidase and lipoxygenase). A complete inactivation of the enzymes was obtained with the blanching temperature of 100°C for 6 min.

Three different food gums (Xanthan, Low Methoxyl Pectin and High Methoxyl Pectin) was used in differing amounts (0.5, 1.5 and 3.0% w/w) to investigate the effects of varying temperature (, 70, 80, 90 and 100 °C) on the rheological properties of chilli puree subjected to the 20 minutes of evaporation process. The rheological characteristics of the puree were evaluated using a Brookfield viscometer at the above mentioned temperatures and it was found that the Power Law model adequately represented shear stress-shear rate data. Temperature dependency of the apparent viscosity at all the shear rate followed the Arrhenius relationship and the flow activation energy ranged between 20.15 and 20.17 kJ/mol for HMP, 22.56 and 28.77 kJ/mol for LMP and 6.54 and less for Xanthan gum. Xanthan gum was found to be the least temperature dependant and contributed to the highest viscosity among all three gums. Xanthan was chosen to be used as the gum to enhance the viscosity of the puree for the final preparation process which involved blanching, pulping, canning and sterilization.

First order reaction kinetics fitted adequately to predict colour loss of the puree during the evaporation process. The colour degradation was found to be independent of the types of gum and their respective concentrations. The individual and interactive effects of process time (15,30 and 60 mins) and concentration (0.5,1.5 and 3.0%) of xanthan gum on rheological characteristics were studied by the application of the response surface methodology during the sterilization process. It was observed that process time individually affected colour degradation, while there was no effect of process time on apparent viscosity and water holding capacity of the puree. The concentration of gum ($P < 0.05$) and process times individually and in combination affected process lethality values.

CHAPTER 1

INTRODUCTION

1.1 General Information

Chillies are members of the genus *Capsicum* and belong to the Solanaceae family along with tomato, potato, eggplant and tobacco. Over 90 species of *Capsicum* have been described by taxonomist, but only 20 are widely recognized. Chillies are high in vitamin content (namely vitamins C, B, E), flavanoid, capsaicin, mineral content and a spice of commercial importance. Chillies are usually processed in the dried form that lacks fresh capsicum colour and flavour (Luning et al., 1995). The powder form of chilli is also adulterated by mixing some colour preservatives to enhance the colour of the powder. As for the locally available "chilli boh", this form of preparation is usually high in acid and preservatives and has a short shelf life. Although methods of preservation such as drying had been widely practised, there are demands for alternate processes, which produce favourable nutritive, physicochemical qualities and shelf-life extensions of chilli-based products. Thermally processed chilli puree and paste are such products that could retain colour and flavour in a semi-solid form, having qualities close to the fresh ones. Chilli puree can be used directly in cooking or food preparations for domestic purposes and bases for sauces and other food production. Thermal processing of chilli and canning in the form of puree is one such process of producing a product with an extended shelf-life, while retaining its favourable qualities. The canning and sterilization process of chilli in the form of chilli puree has the potential of extending the current uses of chilli. The wide spread popularity of thermally processed tomato puree among domestic and industrial use has extended the uses of the vegetable. The current research hopes to develop an alternate product and also expand the versatility of chilli based products in all sectors.

Thermal processing of foods has both positive and detrimental effects on the food. New methods of processing including High Temperature Short Time (HTST), aseptic processing and retort pouches improve the nutritional quality of processed foods. Frequently marketed fresh produce may take several days before sale and eventual preparation results in considerable loss of nutrients. Adequate thermal processing provides convenience as well as a safe food supply to the consumer. In addition, the availability of seasonal foods year round reduces the risk of poor nutrient availability during the off seasons. Canning of food in metal cans has been a widely practiced method in the area of food preservation. While the usage of jars and plastic containers contribute to problems such as seal integrity, aroma sorption, gas permeability and distortion of containers at elevated processing temperatures, canning in metal cans prove to overcome some of the problems mentioned.

Uncontrolled thermal process parameters will undoubtedly have adverse effects on the palpability and nutritional qualities of products processed in metal cans. The quality of foods such as the organoleptic properties (colour, flavour, texture) and nutritional value depends not only on their state before processing and the storage conditions, but also on the extent of changes occurring during thermal operations. Thermal processing conditions that may influence protein denaturation, loss of essential nutrients and deterioration in textural properties have been reported by researchers for products processed in metal cans (Castrillon et al., 1996; Ramaswamy et al., 1997; Rao & Lund, 1986). In chilli puree, colour plays an important role in appearance and acceptability of the product. Some of the pigments responsible for chilli colour are keto-carotenoids, violoxanthin, lutein and β-Carotene. Colour degradation kinetics of food products are complex and need empirical and experimental modelling techniques in order to determine the actual degradation. This phenomenon has led to several researchers (Ahmed et al., 2000; Nisha et al., 2004; Barreiro et al., 1997) exploring the possible kinetic of colour degradation during fruit and vegetable processing. The evaporation process plays an important role in

removing undesired water vapour in order to concentrate the puree. Naturally occurring pectin in vegetables help to maintain viscosity and consistency of products. With the use of food grade gums, a product with improved rheological properties, namely the viscosity is achieved. For vegetables with less pectin, gums are used to contribute towards smoother and more blend flavour in liquid, semi-solid and solid foods (Glicksman & Forkas 1967).

1.2 Research Objectives

The overall objective of the research was to study the effects of thermal processes such as blanching, evaporation and sterilization on the properties of the chilli puree. The specific objectives are:

(i) determine the nutritive and physico-chemical components in *Capsicum Annum var Kulai*

(ii) determine the desirable temperature and time regimes for the blanching process against the destruction of peroxidase and lipoxygenase

(iii) evaluate the effect of varying temperature and gums (High Methoxyl Pectin, Low Methoxyl Pectin and Xanthan Gum) concentration during the evaporation process for 20 minutes on the rheological characteristics of the puree in order to select one gum which gives best results in terms of viscosity and heat stability

(iv) investigate the kinetics of degradation of visual colour using Hunter tristimulus colour scale L, *a* and *b* values and colour pigment (β-Carotene) during evaporation process of the gum selected prior.

(v) determine the process lethality value (F value) for the canned puree subjected to varying consistency (0.5% w/w, 1.5%w/w and 3.0% w/w)

(vi) examine the effect of varying process time (15, 30 and 60 minutes) on the viscosity, colour and water holding capacity and process lethality values of the puree before and after processing

CHAPTER 2

LITERATURE REVIEW

2.1 Origin of chilli

Chillies are members of the genus *Capsicum* and belong to the Solanaceae family along with tomato, potato, eggplant and tobacco. Over 90 species of *Capsicum* have been described by taxonomist, but only 20 are widely recognized. The chilli plant is generally accepted as being native to the western hemisphere. Chiles were used in the pre-Columbian New World to impart flavour and spiciness to food. The Spanish and Portuguese explorers took chillies with them on their travels, and the plant rapidly established itself along the new maritime trade routes to North Africa, the West African coast, Madagascar, and India. The native populations there incorporated chillies into their diets, and chillies soon became a part of the cuisines of those regions. By 1550, Chiles had reached western China, Southeast Asia, and the East Indies (http://www.geocities.com/wstarron/trigeminal response page.html).

2.1.1 Types of chilli

There are various types of local and foreign variety of chillies. Chillies are known by different names in different localities. For example, the poblano chilli is referred to as the pasilla in California and parts of the Southwest. Sometimes different chillies go by the same name because they are similar in appearance. For example, red Kulai variety chillies are sometimes mistakenly labelled as other variety because they are similar in shape, size, and colour. However, the red Kulai is a distinct variety because of its heat. Often, particular varieties of chillies are grown only in particular regions or localities. Usually this is a matter of suitable growing conditions (soil make-up and quality, climate, etc.). Malaysia alone has planted many types of chillies to meet the local as well as export demands of the nation. The common types of chillies

planted by local breeders and their respective physicochemical properties are illustrated in Table 2.1

Table 2.1 Types of local varieties of chilli

Variety of chilli	General characteristics
MARDI MC11 (Capsicum *annum*)	Pungent For processing and fresh consumption purpose
MARDI MC 12 (Capsicum *annum*)	Less pungent Suitable for fresh consumption and processing purposes
CILIBANGI-1(Capsicum *annum*)	Optimum biomass For fresh usage and consumption
CILIBANGI-2 (Capsicum *annum*)	Suitable to be made as dried chilli
CILIBANGI-3 (Capsicum *annum*)	Very pungent
CILIBANGGI–4 (Capsicum *annum*)	Suitable to be made as dried chilli
CILIBANGGI-5 (Capsicum *annum*)	Less pungent Suitable for fresh consumption
CILIBANGGI-6 (Capsicum *annum*)	Pungent Big pod
Kulai (Capsicum *annum*)	Very pungent Longish in shape
Birds eye chilli/Thai chilli (Capsicum *frutescens*)	Extremely pungent Small pod

2.1.2 Uses of chilli

As a food with an excellent source of vitamins A and C, chillies have versatile uses such as in the fresh, dried and processed form. Red chillies also possesses a higher mineral content, surpassing the green pepper in K, Mg, P, Fe, Cu, Zn, Mn and B concentrations (Rubio et al., 2002).

Chillies are used either green or mature, or in preparation of pastes, and as a pungent spice or a condiment because of its colour and flavour. Fresh chillies are consumed widely as an important spice in domestic cooking and food preparation. Similar usage arises for dried chilli as they have a longer shelf-life therefore suitable for storage purposes. The processed form of chilli can be in its unadulterated form

whereby can be used as bases for downstream processes in the food industry. Other options are to add spices and made into sauces. Apart from functioning as a food constituent, chillies are also gaining popularity in the field of pharmacology and therapeutic uses as slimming creams, pain relaxants, ointment and other medicinal balms . All chillies contain capsaicinoids, a natural substance that produce a burning sensation in the mouth, causing the eyes to water and the nose to run, and even induce perspiration. Capsaicinoids have no flavour or odour, but act directly on the pain receptors in the mouth and throat. Capsaicin, the primary capsaicinoid is found primarily in the chilli's placenta, and unevenly distributed throughout the pod wall of the vegetable. Capsaicinoid content is measured in parts per million. These parts per million are converted into Scoville heat units, the industry standard for measuring the pungency level of chillies. Roughly, one part per million chilli 'heat' rates as 1.5 Scoville units.

2.1.3 The import and export of chilli in Malaysia

Figure 2.1 shows the import and export of chilli in Malaysia in metric tonnes. It is clear that even though we produce our own chillies, the huge demand for the vegetable causes rise in the import bill. Chilli (*Capsicum* sp) is a vegetable with high economic importance in the world. Based on the import value and demands in chilli, it is one of the prioritized vegetable by MARDI in conjunction with the national agriculture plan to decrease the import of chillies in the country. Based on the Figure 2.1, the export value of the chilli did not fluctuate much from 1995 to 2001. However, the value did rise steadily from year 1999 to 2001.

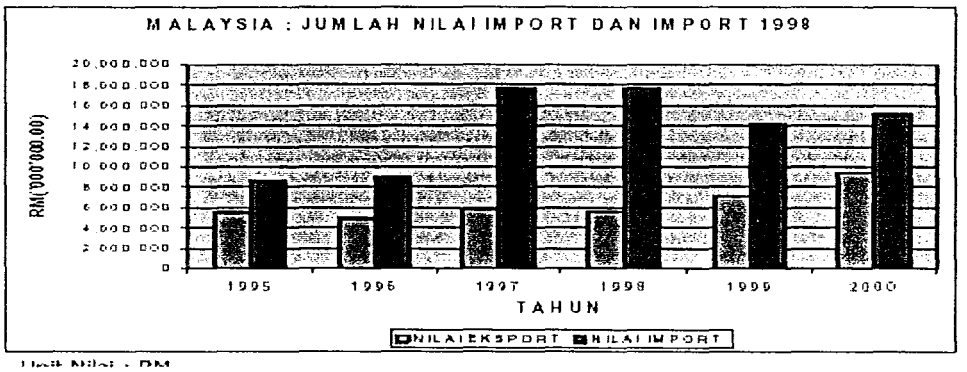

Figure 2.1 Import and export values of chillies
Source: Malaysian Statistic Department, 2001

Chillies are also used widely in the institution and research sector as well as industry for research and production of other food based products (Table 2.2). Meanwhile the figures for the domestic sector depicts average consumption of chilli by Malaysians.

Table 2.2 Amount of chilli usage in the institution, domestic and industry sector

Year	Amount of usage (million tonne)		
	Institution	Domestic	Industry
1991	5,282.30	21,684.40	2,037.00
1992	5,431.70	22,248.20	1,963.00
1993	4,252.10	22,826.70	13,215.00
1994	5,203.90	23,420.20	15,917.30
1995	5,984.50	24,029.10	17,476.80
1996	6,882.20	24,653.90	20,098.40
1997	7,914.50	25,294.90	23,113.10
1998	9,101.70	25,952.50	2,658.10

Source: http://www.agribdc.com/view.php

Malaysian chillies are mainly exported to countries such as Thailand, Brunei, Singapore and the United Kingdom holding an export value of 0.20% in the global market. In peninsular Malaysia, the main states which produce chillies are Perak, Johor and Kelantan. The local chilli market is divided into 3 main categories which are institution, household and industry. The usage of chilli is divided into 4 main categories

which are green chilli, red chilli, dried chilli and 'chilli padi'. It is estimated that for the coming years, the demand for chilli will increase steadily due to the increase in the Malaysian population (http://www.agribdc.com/view.php). The demand will extend to both fresh chillies as well as processed forms such as sauces and paste. This will also increase the use of chillies by the local industry to meet the demands of the consumers.

2.1.4 Pigment composition of chilli

Contributions from about 20 carotenoids control *Capsicum* pod colour. The keto-carotenoids, capsanthin, capsorubin, and cryptocapsin are almost exclusive to the genus *Capsicum*. The major red colour in paprika comes from the carotenoids capsanthin and capsorubin, while the yellow-orange colour is from beta-carotene (β-carotene) and violaxanthin (Reeves, 1987). The colour of the ripe fruit is due to the carotenoid pigments with three keto-carotenoids mainly. The amount of total carotenoids is 30±0.06% capsanthin, 5±0.15% capsorubin and about 5% cryptoxanthin (Minguez- Mosquera & Hornero-Mendez, 1998).

Deli and Toth (1997) have reported that in the ripened fruits of *Capsicum annum* Cv Bovet 4, capsanthin and zeaxanthin accounted for about 45 and 8% respectively of the total carotenoids, respectively, β-carotene and β -cryptoxanthin for about 5 and 3% respectively. Carotenoids are a group of fat soluble pigments that absorb light in the 400-500 nm region of the visible spectrum. This physical property provides the characteristic red/yellow colour of the pigments. They are synthesized by plants to offer protection against sunlight.

Nutritionally, carotenoids are actively involved in the maintenance of the visual system. Hydrocarbon carotenoids are classified as carotenes and xanthophylls. Chemically, the carotenes (C_{40} H_{56}) are highly unsaturated carotenoids and

represented by β-carotene, alpha-carotene, gamma-carotene and lycopene. The hydroxy-keto derivatives of carotenes are called "xanthophylls". It includes lutein, zeaxanthin, cryptoxanthin, capsorubin, capsanthin, astaxanthin, and canthaxanthin (http://www.zealutein.com/Carotenoids.html). Some carotenoids such as β-carotene and cryptoxanthin are converted into Vitamin A in the body. Commercial sources of carotenoids include plant materials and marine algae. Although available from most plant tissues, carotenoids free of other plant pigments are most easily obtained from flowers (marigold), fruits (berries) and root parts (carrots and yellow potatoes), where they are stored in the chromoplasts of the plant cells.

The highly unsaturated conjugated chain of carotenoids is very sensitive to air, oxidizing and reducing agents and structural alterations. Ascorbic acid, alkaline pH, low temperature and darkness were determined to retard degradation of the carotenoids and hydroxylamine was found to inhibit the light reaction (Harkay-Vinkler, 1974). The impact on pigment content varies with species, temperature, and duration of heat-treatment (Paull & Chen, 2000). Physical treatments such as processing, blanching and pulping applied to the fruits or vegetable might alter the enzymatic systems by partial or complete inhibition of their activities, causing changes in the pigment profile.

Processing conditions subjected to high temperature and thermal stress may imply development of degradative reactions related to heating processes such as thermal degradation and isomerization. Examples of those reactions affecting β-carotene content have been described to take place during processing of foodstuffs like tomato products (Shi et al, 1999), canola oil (Goulson & Warthesen, 1999), carrot juices and carotene-containing preparations (Marx et al., 2003). Depending on the time–temperature conditions applied, isomerization and total carotene degradation levels reached on the product were different. Thus, blanching, pasteurization and

sterilization caused mainly trans–cis isomerization while more aggressive thermal processes produced carotene destruction (Perez-Galvez et al, 2004).

2.2 Blanching process

Blanching is one of the most widely used pre-treatment and aims to inactivate enzymes such as lipase, lipoxygenase, phenolase, catalase, peroxidase which cause undesirable flavour, colour and aroma changes in the finished product during storage. Conventional blanching can cause alterations in the structure of food products which lead to an irreversible loss of texture. The optimisation of blanching conditions implies a commitment between keeping the nutritional, organoleptical and structural quality of the food, and its stability during the storage as a result of enzyme inactivation (Luna et al., 1986). Blanching is the process of heating vegetables to a temperature high enough to destroy enzymes present in the tissue. Blanching stops the enzyme action, sets the colour, and shortens the drying and dehydration time. The blanching process aims to inactivate enzymes prior to freezing, to remove tissue gases, to wilt the tissue to facilitate packing, or to cleanse the tissue before canning. Blanching is an operation in which fruit, vegetables, seafood and meat are directly exposed to hot water or steam prior to freezing or canning.

The fundamental aim of blanching is to inactivate enzymes that cause the deterioration of these foods (Kosmala et al., 1984). The effect of blanching on vegetables consists in inhibiting enzyme activity and thus reducing the enzymatic browning effect caused by enzymes such as peroxidase, lipoxygenase and chlorophyllase, blocking the development of the foul smells for which lipoxygenase and protease are responsible, stabilizing the nutritional value of the product, preventing the oxidating activity of ascorbic acid (Barrett & Theerakulkait, 1995; Frost, 1992; Sheu & Chen, 1991). Conventional blanching equipment uses water and energy to constantly produce hot water or steam. Blanching stops the enzyme action which causes loss of

colour and flavour during drying and storage, improves the colour, and shortens the drying and rehydration time by relaxing the tissue walls so moisture can escape or re-enter more rapidly. Blanching is usually done in hot water or in steam. In water blanching, the product is submerged in water while in steam blanching, the product is carried on a wire mesh belt through a hooded section where steam is injected. By controlling the time and temperature of the process, enzymes are inactivated throughout the product. The duration and temperature of the blanching treatment are chosen so that the excess enzyme load can be inactivated without any unnecessarily prolonged and costly heating of the product. Song et al. (2003) have concluded that high temperature short time regime of blanching process for vegetable soybeans resulted in minimal loss of nutrients and vitamins.

2.3 Enzyme deactivation

2.3.1 Peroxidase (POD)

2.3.1.1 Introduction

Peroxidase (POD) has relatively high resistance to thermal inactivation and is extensively distributed in plant tissues causing it to be used as an index of enzyme activity in plant tissues. The activity of peroxidase causes deteriorative changes in vegetable tissues and it has been generally accepted that if peroxidase is destroyed by a given heat treatment, it is unlikely that any other enzyme system would have survived. Peroxidase isolated from the same plant may exhibit different properties and possibility of multiple forms of the enzyme to occur is also high. Peroxidases are quite specific in their primary reaction with peroxides. Peroxidase appears to be most stable at pH 7, where they also exhibit maximum catalytic activity (Adams, 1997). Peroxidase promotes a variety of consequential reactions during the coupled reactions whereby primary oxidation products react with secondary substrates (Gkinis & Fennema, 1978). In vegetables, peroxidase is located in soluble form in the cell cytoplasm, and in

insoluble form as ionically bound and as covalently bound to the cell wall (McLelland & Robinson, 1981; Vamos-Vigyazo, 1981).

It was suggested that peroxidase plays a role in the degradation of colour pigments in vegetables. Walker (1964) suggested that peroxidase plays a role in chlorophyll degradation while Kampis et al. (1984) proposed that soluble peroxidase is responsible for colour changes in frozen green vegetables during long storage. In addition to that, a carotene bleaching system related to peroxidase activity was observed in the water soluble fraction of tomato extracts (Blain et al., 1968) and in the protein fraction of red pepper extracts (Kanner et al., 1977).

2.3.1.2 Mechanism of peroxidase reaction

Peroxidases reduce H_2O_2 to water while oxidizing a variety of substrates. Thus, peroxidases are oxidoreductases which use H_2O_2 as electron acceptor for catalyzing different oxidative reactions. The overall reaction is as follows (Maehly, 1960):

donor + H_2O_2 <=> oxidized donor + 2 H_2O

2.3.1.3 Inhibition and inactivation of peroxidase

The inhibition and inactivation of peroxidase occurs during heat treatment. Heat inactivation of POD generally occurs in biphasic stages due to the presence of isoenzymes with different thermal stabilities (Gune & Bayındırlı, 1993; Morales-Blancas et al., 2002). Three main processes have been considered to be involved in the inactivation of peroxidase; (1) dissociation of prosthetic (heme) group from the haloenzyme (active enzyme system); (2) conformation change in the apoenzyme (protein part of the enzyme); and/or (3) modification or degradation of the prosthetic group (Lemos et al., 2000). Other methods of would include using new technologies to reduce the intensity of heat treatment such as ultrasound (Cruz et al., 2004) and microwave treatment (Soysal & Soylemez, 2005).

2.3.2 Lipoxygenase (LOX)

2.3.2.1 Introduction

Lipoxygenases (LOX), formerly known as lipoxidase or carotene oxidase, are an iron containing dioxygenase which catalyses the oxidation of polyunsaturated fatty acids. A lipoxygenase definition according to enzyme classification is linoleate: oxygen oxidoreductase (for plant lipoxygenase) and arachidonate: oxygen oxidoreductase (for mammalian lipoxygenase). Lipoxygenase are predominantly classified according to their positional specificity of the dioxygenation of their most common substrates linoleate (C-18) in plants, and arachidonic acid (C-20) in mammals (Brash, 1999).

Lipoxygenase is widely distributed in nature, being found in essentially higher plants and animals. These multiple form enzymes exist in seeds, legumes, green parts and fruits of a plant, mushroom (*Agaricus bisporus*) (de Lumen et al., 1978) and in lower forms of plants such as *Chlorella pyrenoidosa* (Zimmerman & Vick, 1973). Lipoxygenase from different sources, as well as their isoenzymes, may differ in substrate specificity, optimum pH, end-products produced, heat stability and the ability to participate in co oxidation reactions (Wong, 1995). Two major categories of plant lipoxygenase which exist are type one lipoxygenase which exist in soybean have pH optima of 9 and lower tendency to participate in co-oxidation in other words oxidises only free fatty acids. Type two lipoxygenase have pH optima of 6.5 and attacks oils and fats which will yield both 9- and 13-hydroperoxides (http://www.dkfz-heidelberg.de/spec/lox-db/lipoxygenase-info.php). Table 2.3 shows the properties of lipoxygenase in various vegetablse and fruits.

Table 2.3 Occurrence and properties of various lipoxygenases

Food	pH optimum	Peroxidation specificity		Type
		9-LOOH(%)	13-LOOH(%)	
Soybean, L-1	9.0	5	95	I

Soybean, L-2	6.5	50	50	II
Pea L-2	6.5	50	50	II
Peanut	6.0	0	100	I
Potato	5.5	95	5	I
Tomato	5.5	95	5	I
Wheat	6.0	90	10	I
Cucumber	5.5	75	25	-
Apple	6.0	10	90	-
Strawberry	6.5	23	77	-
Gooseberry	6.5	45	55	II

2.3.2.2 Mechanism of lipoxygenase reaction

Figure 2.2 below illustrates the model of the catalytic cycle of the lipoxygenase. Lipoxygenase has an iron (Fe) atom in its active centre and is usually in the inactive ferrous state. The iron atom is involved in electron transfer during the incorporation of oxygen into unsaturated fatty acids containing cis,cis-1,4-pentadiene systems (Hilderbrand,1989).

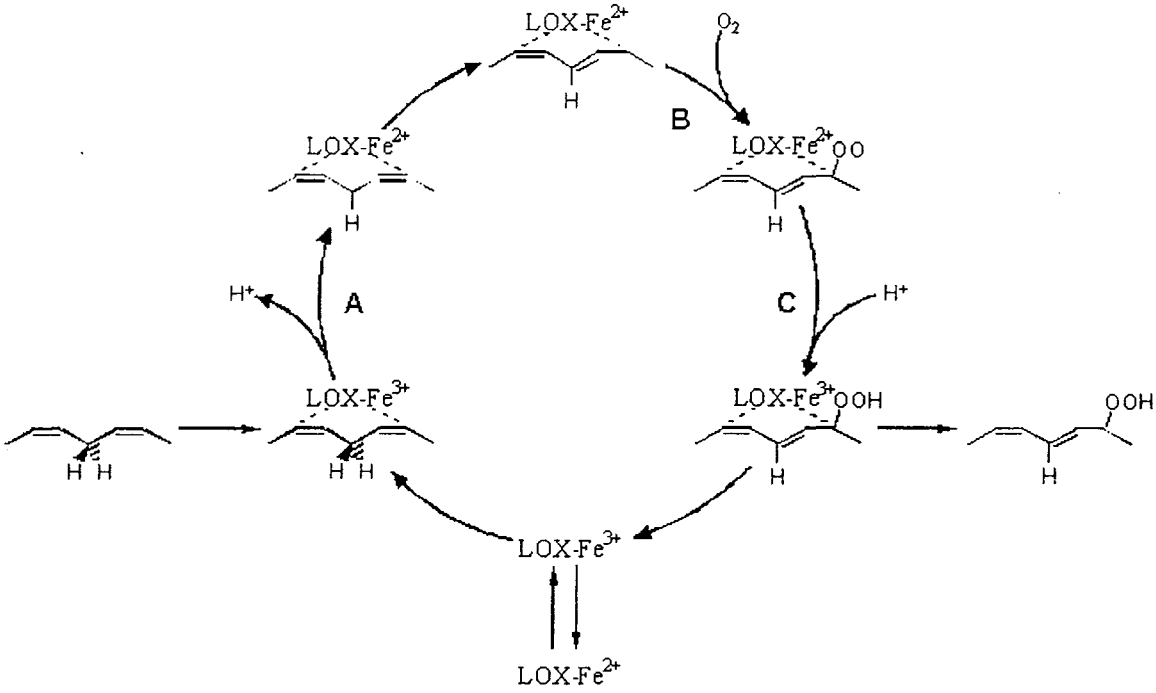

Figure 2.2 Model of the catalytic cycle of LOX.
Source *http://www.dkfz-heidelberg.de/spec/lox-db/lipoxygenase-info.php*

Lipoxygenase from different sources tend to generate different amounts of the 9- and 13-hydroperoxides. In tomato, lipoxygenase yields 9, 12, 13, 16 hydroperoxides

and oxidation of these hydroperoxides then yield primarily 9, 13 hydroperoxide at a ratio of 95:5 respectively (http//: www.cmbl.org.pl/vol8/V8Page279.pdf).

2.3.2.3 Inhibition and inactivation of lipoxygenase

Lipoxygenase participate in reactions of biochemical transformations of lipid fraction. As a result of lipoxygenase activity, the hydroperoxides derivatives generated undergo further rearrangements, be degraded to secondary products and react with other compounds leading to production of off-flavours, fat deterioration and lowering nutritional value of food stuffs (Kubicka et al., 2000). In soybean products, volatile degradation of fatty acids (linoleic and linolenic acid) has been associated with grassy-beany and rancid off-flavours which reduce the consumer acceptability (Rackis et al., 1979). Kazeniac and Hall (1975) have reported that lipoxygenase generated cis-3-hexenal, n-hexenal and trans-2-hexenal resulted in the development of 'green' and rancid off-flavours in canned tomato juice. Williams et al. (1986) evaluated the sensory character of blanched vegetable purees to which isolated enzymes had been added and found that lipoxygenase was the enzyme most active in aroma deterioration in English green peas and green beans.

Besides having the off-flavour characteristics, lipoxygenase will also oxidise carotenoids and chlorophyll and thus degrade them to colourless products, a property used in flour "bleaching". This co-oxidation of plant pigments namely carotenoids, chlorophyll, cantaxanthin and other pigments happen in a lipoxygenase system under both anaerobic (Type 1 lipoxygenase) and aerobic (Type 2 lipoxygenase) system. Due to the nature of the enzyme, inhibition of lipoxygenase is undertaken by incorporating various antioxidants into the system. However, the ability to control lipoxygenase activity is limited by the difficulty of introducing them into plant tissues prior to disruption of the cells (Eskin et al., 1977). Other methods of inhibition include heat treatment and pH adjustment.

2.4 Gums and stabilizers

2.4.1 Introduction

Gums are water-soluble, high molecular weight polysaccharides that serve a variety of functions in food systems, such as enhancing viscosity, creating gel-structures, formation of a film, control of crystallization, inhibition of syneresis, improving texture, encapsulation of flavours and lengthening the physical stability (Dickinson, 2003; Dziezak, 1991). They are integral ingredients in fluid foods used for controlling viscosity and mouth feel. Gums are used in foods primarily as thickeners and gelling agents as a result of their ability to alter the rheological properties of the solvent in which they are dissolved. These functional ingredients are widely used in dairy products, canned foods, bakery products, salad dressings, beverages, sauces, soups and other processed foodstuffs to improve textural characteristics, flavour and shelf life. Several authors have reviewed various applications of food hydrocolloids in the food industry (Anderson & Andon, 1988; Ward, 1997). The change in viscosity occurs as a result of the high molecular weight polymeric nature of the gums and the interactions between polymer chains when gums are dissolved or dispersed (Yaseen et al., 2005). Some examples of gums used widely in food industries are carrageenan, microcrystallinecellulose, konjac, gum arabic carboxymethylcellulose, xanthan, locust bean , guar and pectin. Due to the difference in gum structure and extrinsic conditions within the fluid food system, the rheological behaviour is quite different from one gum solution to another.

2.4.2 Pectin

2.4.2.1 Structure

Pectins are polysaccharide in the cell walls of higher plants and are very important in food applications as a gelling agent, thickener, texturizer, emulsifier, and stabilizer. Pectin is a linear polysaccharide built from polygalacturonic acid subunits

that is partially esterified with methoxyl groups. Pectin consists essentially of linear chains of α-D galacturonic acid residues with a small fraction of rhamnose and small side chains formed by other sugars (Van Buren, 1991). Pectin is usually characterized by the molecular size, the degree of methoxylation, pH, and ionic strength. The degree of methoxylation is very important and is defined as the average number of methoxyl groups per percent of the galacturonic acid units. This is commonly referred to as the degree of methoxylation (DM) and used to classify pectins into two groups. If the DM is greater than 50%, the pectin is referred to as high methoxyl pectin (HMP). Pectins with less than 50% DM are called low methoxyl pectin (LMP). When pectin is extracted, much of the hairy regions are destroyed, leaving mainly the smooth galacturonic acid regions, with a few neutral sugar units attached or in the main linear chain. The nature and placing of these neutral sugars may vary with the source material, and have some influence on the properties of pectins from different origins. However, the biggest influence on pectin properties is the degree of esterification (DE), which determines, for example, the degree of reactivity with calcium and other cations (http://www.ippa.info/what_is_pectin.html).

2.4.2.2 Production

The traditional raw material for pectin production includes apple , squeezed sugar-beet, citrus fruit peel (Bocco et al., 1998), sunflower head and burdock (*Arctium sp.*) (Mkrtchian et al., 1998). The enzyme pectinmethylesterase (PME, EC: 3.1.1.11) has been found in plants, as well as in pathogenic fungi and bacteria (Giovane et al., 1994), and catalyses the hydrolysis of the methyl ester groups of pectin. After this treatment, the pectin can be further hydrolysed through the action of polygalacturonase.

2.4.2.3 Application

At a high concentration, pectins form a gel. In the gelation process, there are substantial different mechanisms between low- and high-methoxyl pectins (Thakur, 1997). Pectin has many applications in food and pharmaceutical industries (Hoefler, 1991; Jongen, 1987; Pilgrim, 1991; Thakur, 1997). In foods, pectin is mostly used in jams and jellies as a gelling agent and thickener. It is also used in drinks, savoury sauces, syrups and some other foods to make a desirable texture (Hoefler, 1991; Pilgrim, 1991).

In low-methoxyl pectins (LMP), gelation results from the ionic linkages via calcium bridge between two carboxyl groups belonging to different chains in close contact with each other to form a structure like an egg box over a wide range of soluble solids and pH values (Grant et al., 1973). In practice, LMPs are gelled by using soluble calcium salts, which can be naturally present in the fruit or milk; or added as a dilute solution. LMP uses are varied, ranging from reduced sugar jams to soft confectionery jellies and spread able processed cheeses. It is well known that the gel strength increases with decreasing temperature and increasing Ca concentration (Clark & Farrer, 1996).

In high-methoxyl pectins, the cross-linking of pectins involves contributions from hydrogen-bonding and hydrophobic interactions between the ester methyl groups in the different molecules (Oakenfull & Scott, 1984).

2.4.2.4 Nutritional and Safety aspects

Pectin is a fine organic powder and a component of the normal diet. It is an approved food additive and ingestion of pectin at reasonable levels is safe. The acceptable daily intake (ADI) for pectins and amidated pectins was established as "not specified" by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) in 1981. The Committee had found that there were no toxicological differences between

pectins and amidated pectins and there were no limitations for the use of pectins from a toxicological point of view. The United States Food and Drug Administration (US FDA) have affirmed that pectins are "generally recognized as safe" for use in human foods under 21 CFR 184.1558. (http://www.cfsan.fda.gov/food_additive.html). This affirmation became effective in 1983 and states that pectin is generally recognized as safe for food use when it is used as emulsifier, stabilizer or thickener and when levels do not exceed current good manufacturing practices (GMP). Under the CODEX general standards for food additives, pectin has also been categorized as additives permitted for use in food in general, unless otherwise specified, in accordance with GMP (Codex Alimentarius Commission., 2002).

2.4.3 Xanthan gum

2.4.3.1 Structure

Xanthan gum consists of 1,4-linked B-D-glucose residues, having a trisaccharide side chain attached to alternate D-glucosyl residues (Jansson et al., 1975). The unique properties of xanthan gum are attributed to its molecular structure. It consists of repeating five sugar residue units, namely two glucose, two mannose, and one glucuronic acid. The polymer backbone resembles cellulose with α-1,4 linked D-glucose. Furthermore, xanthan gum has a trisaccharide side chain attached to every other anhydroglucose unit (Hill, 1985). The xanthan gum molecule undergoes a thermally induced order-disorder conformational transition. The disordered form is favoured by low salt concentrations and high temperatures (Cheetham & Mashimba, 1992). Most publications give evidence that the ordered conformation of xanthan is double-stranded or dimeric (Hjerde et al., 1994).

2.4.3.2 Production

Typically, *Xanthomonas campestris* is cultured in a well-aerated and well-agitated fermenter. The medium contains a carbohydrate source, such as glucose, a

suitable nitrogen source and nutrient salts (Bresolin et al., 1998). When the fermentation has finished, the broth is heated to kill the bacteria and the xanthan gum is recovered by precipitation with isopropyl alcohol. Then the polymer is dried, milled and packaged.

2.4.3.3 Properties

Xanthan gum is a high molecular weight polysaccharide which forms a viscous water solution at low gum concentration. These solutions exhibit small fluctuations in apparent viscosity with changes in environmental conditions such as pH, ionic strength, temperature and presence of enzymes. The gum is soluble either in hot or cold water, has a high viscosity at low concentrations (Alexander, 1999) and shows excellent stability in heat and acid systems (Casas et al, 2000).

Like most other hydrocolloids, xanthan gum needs intensive agitation upon introduction into the aqueous medium in order to avoid the formation of lumps. Xanthan gum solutions are non-Newtonian and highly pseudo plastic. The apparent viscosity changes significantly when different shear stresses are applied-the higher the shear, the lower the viscosity (Oviatt & Brant, 1993). The marked shear-thinning behaviour of xanthan gum solutions may be explained by the conformational status of the polymer molecules. Domains of associated xanthan gum chains exist at rest or at low shear, which are stabilized by hydrogen bonds (Cuvelier & Launay, 1986). On shearing the extent of aggregation is reduced resulting in a lower solution viscosity. This pseudo plasticity enhances sensory qualities (flavour release, mouth feel) in food products and guarantees a high degree of mixability, pumpability and pourability.

The three-dimensional network formed by the chains makes xanthan gum an efficient stabilizer for suspensions and emulsions. In order to adjust the desired flow behaviour xanthan gum is often used in combination with other hydrocolloids. Xanthan

gum interacts synergistically with galactomannans, e.g. locust bean gum and guar gum and glucomannans like konjac mannan. The content of galactose and the distribution of galactose residues in the galactomannan can have a significant influence on the interaction properties with xanthan gum molecules (Tako, 1991). Solution of xanthan obtained by dissolution at moderate temperature tends to be highly viscous. The gum exhibits pseudoplasticity with high viscosity at low concentration (Fox, 1997; Urlacher & Noble, 1997). It has been reported that concentration has considerable effect on rheological characteristics of xanthan gum while temperature and pH did not (Garcia-Ochoa et al., 2000; Marcotte et al., 2001). Synergic effects of xanthan gum with other non-gelling polysaccharides of the galactomanan family have been practiced by the food industries to increase the gross viscosity and gelling properties (Casas & Garcia-Ochoa, 1999; Wang, et al., 2002), however, xanthan has no gel forming ability individually (Williams et al., 1991).

2.4.3.4 Application

Due to the three-dimensional network formed by the associated xanthan gum chains, oiling off and separation of insoluble solid particles is prevented. Further, the shear-thinning flow behaviour contributes to mixability, pumpability and pourability of industrially produced dressings and sauces. Many of today's prepared foods, semi-prepared foods and convenience foods would not be possible without stabilizers and thickeners. In order to adjust the desired flow behaviour, xanthan gum is often used in combination with other hydrocolloids.

Xanthan gum induces cooking and cooling stability of wheat flour and improves the freeze-thaw stability in starch-thickened frozen foods (Rosell et al., 2001). It can also induce dough strengthening; it increases water absorption and the ability of the dough to retain gas, the specific volume of the final bread and the water activity of the crumb (Collar et al., 1999). In desserts, toppings, as well as in dairy products, xanthan

gum is used as a stabilizer, generally in combination with other hydrocolloids. In beverages, xanthan gum is effective for suspending fruit pulp for long periods of time (Genovese & Lozano, 2001). This gives the drink enhanced mouth feel with full-bodied taste and good flavour release. This is especially interesting for low-calorie drinks where sugars are totally or partially replaced by artificial sweeteners, resulting in a 'thinner' consistency. Hydrocolloids are the largest group of additives used in reduced and no-fat convenience foods. Many low-fat food systems use the water-binding abilities of xanthan gum. For example, xanthan gum has been shown to provide moistness in low-fat muffins (Hippleheuser et al., 1995). In low-fat frankfurters, xanthan gum considerably reduced the reheating losses. Low-fat salad dressings will contain a polysaccharide like xanthan gum to increase the viscosity of the aqueous phase and so stabilize the system. The final product has high yield value (permitting the suspension of spices, herbs and vegetables and enabling the dressing to cling to the salad as well as to have body) and strong pseudo plasticity (Nussinovitch, 1997).

Further examples for the use of xanthan gum in low-fat products are the following: mayonnaise, bakery fillings, processed cheese, spreads, dairy products, ready-to-prepare meals.

2.4.3.5 Nutritional and Safety aspects

In 1961, the initial important research publications on the production of xanthan gum appeared. The research laboratories of the US Department of Agriculture discovered that the bacterium *Xanthomonas campestris* found on cabbage plants produces an extra cellular polysaccharide with exceptional rheological properties. Since then, a number of improvements in polysaccharide manufacture have been made. Today, xanthan gum is the most important microbial polysaccharide commercially. Prior to its approval as a food ingredient, xanthan gum had been extensively investigated with respect to toxicology and safety. In 1969 it was cleared

as a food additive by the FDA and registered in the Code of Federal Regulations for use as a stabilizer and a thickener (http://www.cfsan.fda.gov/food additive.html). In 1980 the European Commission (EC) approved xanthan gum under the number E415 in the list of permitted thickeners and stabilizers. In 1988, the Acceptable Daily Intake (ADI) of xanthan gum was altered into 'not specified', thus confirming its status of a safe food additive.

2.5 Thermal processing

2.5.1 Introduction

One way of extending the shelf life of a variety of food products is thermal processing; that is, exposure of the product at elevated temperatures for a relatively short period of time. The objective of the thermal process is to achieve microbiologically stable food product. Thermal sterilization of pre-packaged canned foods in retorts has been one of the most widely used methods of food preservation during the twentieth century and has contributed significantly to the nutritional well-being of much of the world's population. This heat treatment can be used as the single preserving technique (commercial sterilization) or it can be used as one step in conjunction with other preserving factors or processes such as blanching and pasteurization.

Commercial sterility is defined as: the condition achieved by application of heat which renders the food free from viable micro-organisms, including those of known public health significance, capable of growing in the food at the temperatures at which the food is likely to be held during distribution and storage. The extent of the heat treatment will vary depending on the specific objectives concerning the preserving action of the heat treatment and the nature of the product. The microbiological safety of low-acid canned foods depends primarily upon the care and accuracy with which the entire process is carried out. Low-acid foods with a pH of greater than 4.6 must be

processed to commercial sterility. There are two types of spoilage that must be considered, one of significance to public health and the other of commercial significance. From a food safety point of view, in designing thermal processes, the acceptable remaining microbial population which does not impose a public health hazard must be determined in advance.

2.5.2 Microbiology of Canned food

There are two basic approaches to the validation of thermal processes. Traditionally the approach has been to use direct kill measurements involving spore suspensions with known heat destruction/resistance characteristics or other microbial challenge tests. Direct kill methodologies involve the inoculation of sample packs with spore suspensions or the like, or the use of inoculated food particles, e.g. food alginate particles, which have specific application in UHT systems (Gaze & Brown, 1990). It must be realized that a condition of 'zero survivors' is unattainable (in view of the logarithmic nature of microbial destruction) and, therefore, absolute safety is impossible.

It is somewhat intuitive that, in quantifying the microbial requirements in terms of adequate processing time at some appropriate constant temperature, the most heat resistant organism that is expected to be present in the food under consideration as well as the initial load of that organism in the product must be considered; the heat resistance of the organism may be different in different foods. As mentioned earlier, acceptable, remaining, concentrations of non-pathogenic (mesophilic or thermophilic, spore-forming) organisms must also be set in order to minimize economic losses due to product spoilage. So, processes should be designed based on the strictest requirements resulting from safety and economic considerations. As a starting point, a 10^{-12} reduction of the initial concentration of *Clostridium botulinurn* is considered as the minimum process requirement for low-acid foods (Pflug, I987). As far as food safety is